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Article

Integrin $\alpha_5\beta_1$ and Fibronectin Regulate Polarized Cell Protrusions Required for *Xenopus* Convergence and Extension

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Summary

Background: Integrin recognition of fibronectin is required for normal gastrulation including the mediolateral cell intercalation behaviors that drive convergent extension and the elongation of the frog dorsal axis; however, the cellular and molecular mechanisms involved are unclear.

Results: We report that depletion of fibronectin with antisense morpholinos blocks both convergent extension and mediolateral protrusive behaviors in explant preparations. Both chronic depletion of fibronectin and acute disruptions of integrin $\alpha_5\beta_1$ binding to fibronectin increases the frequency and randomizes the orientation of polarized cellular protrusions, suggesting that integrin-fibronectin interactions normally repress frequent random protrusions in favor of fewer mediolaterally oriented ones. In the absence of integrin $\alpha_5\beta_1$ binding to fibronectin, convergence movements still occur but result in convergent thickening instead of convergent extension.

Conclusions: These findings support a role for integrin signaling in regulating the protrusive activity that drives axial extension. We hypothesize that the planar spatial arrangement of the fibrillar fibronectin matrix, which delineates tissue compartments within the embryo, is critical for promoting productive oriented protrusions in intercalating cells.

Introduction

The extracellular matrix (ECM) plays a major role in the development of metazoans. Many ECM molecules function as adhesive ligands and can signal through transmembrane receptors such as integrins and syndecans [1] or can act as scaffolds that bind and restrict the diffusion of growth factors involved in attracting, repelling, or guiding cells during their migrations [2, 3]. One component of the ECM, fibronectin (FN), and the integrin receptors that bind it play central roles in morphogenesis [4, 5]. In FN-deficient mice, gastrulation appears to proceed normally in anterior tissues but fails posteriorly [6]. Similarly, the phenotypes of embryos lacking integrin α_5, the predominant FN receptor, are generally less severe than the FN null phenotype [7, 8]. However, in both cases axial extension is significantly reduced, indicating that more subtle defects in gastrulation movements may be responsible. Additional evidence supporting a critical role for integrin and FN in gastrulation comes from work on salamanders [9–12] and more recently the frog *Xenopus laevis* [5, 13–16]. Thus, integrin-FN interactions participate in a variety of processes that precede the cell movements responsible for convergence and extension and tissue migration. What has not been addressed by these studies is the relationship between integrin-FN engagement and the regulation of mediolateral cell intercalation behaviors that drive convergence and extension.

The narrowing and coordinate lengthening, or convergent extension, of embryonic tissues by cell intercalation is a recurring theme in chordate and invertebrate development [17] with many pathways involved in its regulation. Convergent extension of the dorsal mesoderm in *Xenopus* is driven largely by mediolateral intercalation of postinvolution mesodermal cells [18] as cells extend polarized tractive protrusions at their medial and lateral ends [19]. The cell movements and molecular pathways that regulate convergence and extension have been the subject of intense research in recent years (for reviews, see [20–25]). Recently, components of the extracellular matrix and their cell-surface receptors have been implicated in this behavior [13, 16, 26].

A key question is whether the requirement for cell-FN interactions at gastrulation reflects an adhesive/mechanical role that promotes cell traction and intercalation, or whether the matrix provides instructional information that permits the establishment of polarized protrusive behaviors. In this study, we provide evidence that a FN matrix and signaling through integrin $\alpha_5\beta_1$ are required for productive, oriented protrusions that drive mediolateral cell intercalation and convergent extension.

Results

Fibronectin Protein "Knockdown" Retards Gastrulation and Blocks Convergence and Extension

We repressed FN protein synthesis with antisense morpholino oligonucleotides directed against the two FN genes expressed in the early embryo [27]. FN levels were reduced by 85% to 95% (n = 3; 10 embryos per sample per experiment) relative to control embryos when coinjected with 6.0 to 7.5 μMoles of each antisense morpholino (FNMO; Figure 1A). Patterning of axial mesoderm was unaffected, whereas convergence of the dorsal axial mesoderm was reduced by 20% as shown by a wider region of chordin expression (arrows in Figure 1B), which marks the prospective notochord.

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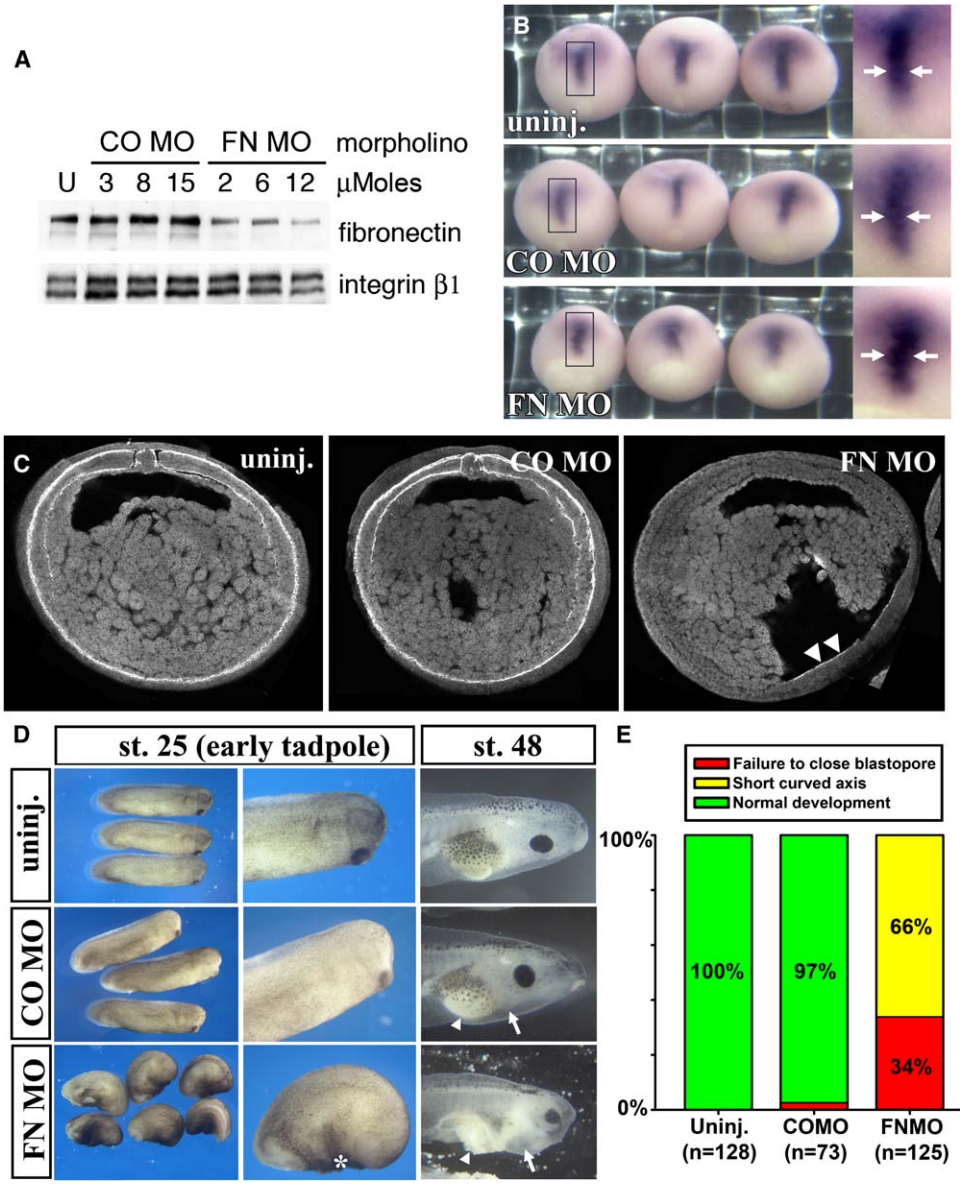

Figure 1. FN Antisense Morpholino "Knocks down" FN Protein Synthesis, Reduces Fibril Formation, and Reduces Dorsal Axis Extension

(A) Western blot analysis of morpholino (MO)-injected embryos harvested at stage 10.5 and processed for detection with the anti-FN mAb 4H2 and the anti-integrin β_1 PcAb 363. The two FN bands correspond to two major alternatively spliced FN subunits expressed during development. The control MO has no affect on the levels of expression of either FN or integrin β_1 at up to 15 μMoles MO injected per embryo. A dose-dependent reduction in FN protein expression is noted in the presence of the combined (50:50) FNMOs. Levels of integrin β_1 protein expression are unaffected by the FN antisense MO.

(B) Expression of chordin, a marker of dorsal axial tissues, shows that embryos injected with 15 μMoles FNMO have wide dorsal axial tissues. (Arrows mark the width of the chordin expression.)

(C) Transverse confocal sections of embryos stained for FN fibrils show a typical pattern in both control morpholino and uninjected control embryos while fibril formation is almost completely abolished (see arrowheads for an example of faint labeling of blastocoel roof ectoderm in FNMO-injected embryos).

(D) FNMO-injected embryos show defects in both dorsal and ventral elongation by early tadpole stages and severe anterior mesoderm defects by later tadpole stages. At early tadpole stages, the dorsal axial extension is moderately reduced while ventral extension is severely reduced (asterisk). Late-stage FNMO-injected tadpoles show defects in ventral mesoderm morphogenesis, ectodermal lesions, and failure of heart (arrow) and gut (arrowheads) formation.

(E) Pooled data from three batches of embryos were observed over several days and their progress was assessed. None of the embryos injected with 15 μMoles FNMO develop beating hearts.

FNMO-injected embryos exhibit a near complete absence of FN fibrils (some fibrils are still evident on the walls of the blastocoel; Figure 1C, arrowheads), and when cultured to later stages (Figure 1D), embryos exhibit similar phenotypes (Figure 1E) to those observed after blastocoelic injection of anti-FN or anti-integrin $\alpha_5\beta_1$ function-blocking antibodies or expression of integrin β_1 dominant-negative constructs [13, 14, 28–30].